AlphaLISA® *SureFire® Ultra™*

Human p-IRF7 (Ser477) Detection Kit

Product number: ALSU-PIRF7-A500, ALSU-PIRF7-A10K, ALSU-PIRF7-A50K, ALSU-PIRF7-A-HV

Kit specificity:

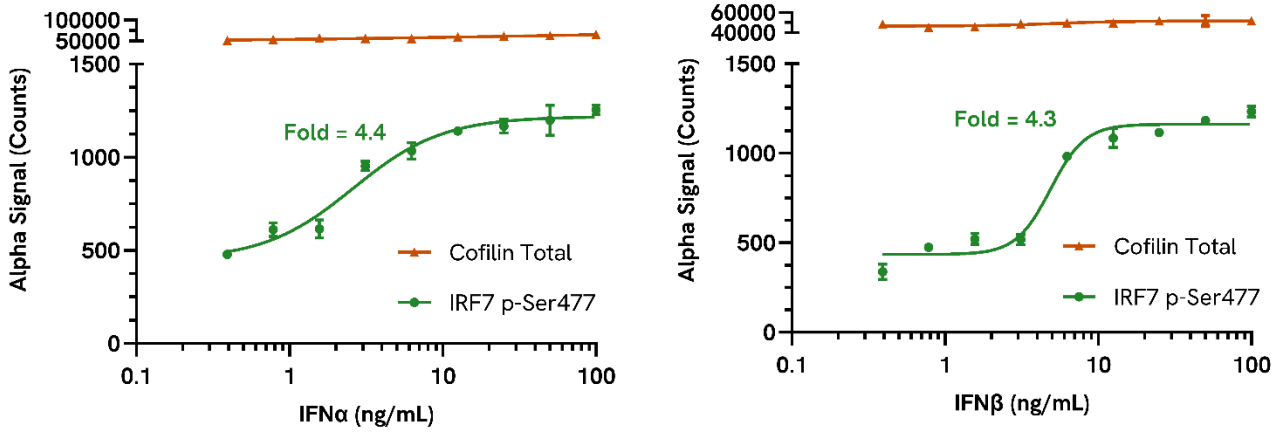
This assay kit contains antibodies which recognize the phospho-Ser477 epitope and a distal epitope on IRF7. The protein detected by this kit corresponds to UniProt ID Q92985. IRF7 is also known as interferon regulatory factor 7. These antibodies recognize IRF7 of human origin. Other species should be tested on a case-by-case basis.

Control lysate information:

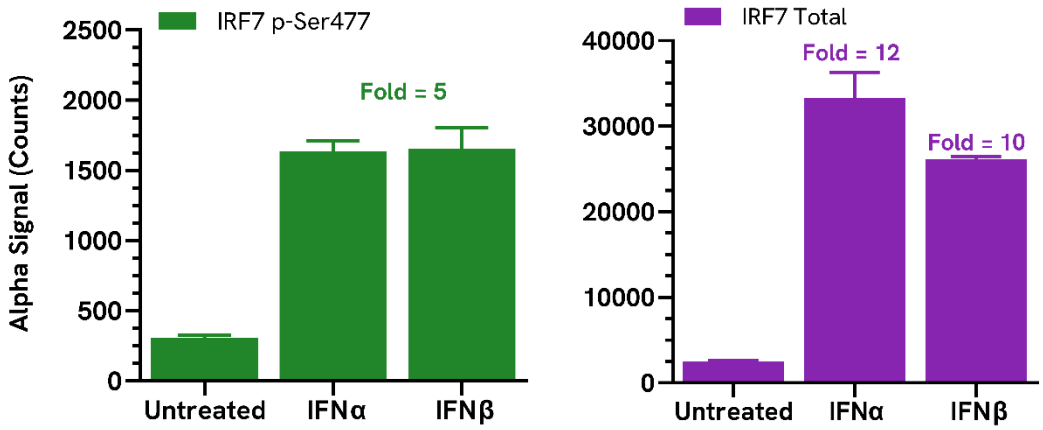
Positive Control Lysate: Prepared from THP-1 cells seeded at 500K cells/mL in medium containing 100 nM PMA and incubated for 24 hours. THP-1 derived macrophages were treated with 100 nM Calyculin A for 30 minutes, washed with HBSS and lysed in Lysis Buffer at a final concentration of 8 x 10^6 cells/mL.

Representative data:

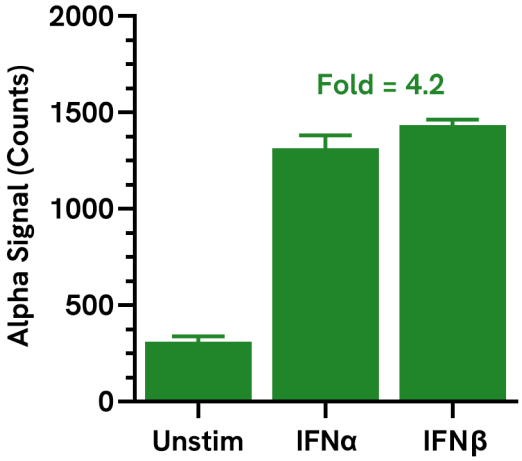
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded at 400K cells/well in complete medium and treated with IFNα or IFNβ at the indicated concentrations for 6 hours. Cells were washed with HBS and lysed with Lysis Buffer. Lysates were assayed separately for Phospho (Ser477) IRF7 and Cofilin Total using respective *SureFire Ultra* assay kits. Equivalent to approximately 80,000 cells/datapoint for Phospho (Ser477) IRF7 and 8,000 cells/datapoint for Cofilin Total.



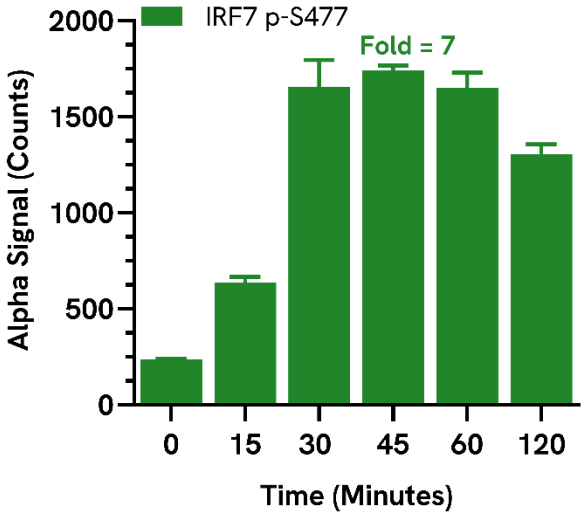
Data obtained with a 2-plate, 2-incubated protocol. THP-1 cells were seeded at 100K cells/well in medium containing 100nM PMA and incubated for 24 hours. THP-1 derived macrophages were then treated with 250 ng/mL of IFNα or IFNβ for a further 24 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* assay kits. Equivalent to approximately 10,000 cells/datapoint.



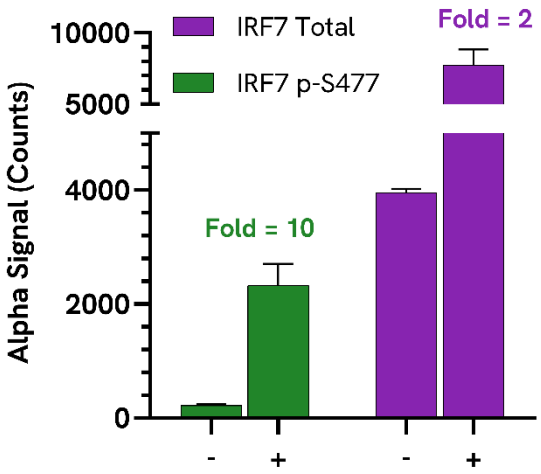
Data obtained with a 2-plate, 2-incubated protocol. HT 29 cells were seeded at 20K cells/well and incubated overnight. Cells were treated with 100 ng/mL of IFNα or IFNβ for 24 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed for Phospho (Ser477) IRF7 using the *SureFire Ultra* assay kit. Equivalent to approximately 2,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubated protocol. THP-1 cells were seeded at 400K cells/well in complete medium and treated with 20 µM of STING agonist, diABZI at the indicated time points. Cells were washed with HBSS, lysed with Lysis Buffer and assayed for Phospho (Ser477) IRF7 using the *SureFire Ultra* assay kit. Equivalent to approximately 40,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubated protocol. THP-1 cells were seeded at 400K cells/well in complete medium and treated with 100 µg/mL of STING ligand, 2'3' cGAMP for 4 hours. Cells were washed with HBSS, lysed with Lysis Buffer and assayed separately for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* assay kits. Equivalent to approximately 40,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubated protocol. THP-1 cells were seeded at 100K cells/well in medium containing 100 nM PMA and incubated for 18 hours. THP-1 derived macrophages were then treated with Calyculin A at the indicated concentrations for 30 minutes. Cells were washed with HBSS, lysed with Lysis Buffer and assayed for Phospho (Ser477) and Total IRF7 using respective *SureFire Ultra* assay kits. Equivalent to approximately 10,000 cells/datapoint.